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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/970,382	10/03/2001	Su-Chun Zhang	960296.98211	9657

27114 7590 11/19/2002

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EXAMINER	
NGUYEN, QUANG	

ART UNIT	PAPER NUMBER
1636	9

DATE MAILED: 11/19/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/970,382	Applicant(s) ZHANG ET AL.	
	Examiner Quang Nguyen, Ph.D.	Art Unit 1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *26 August 2002* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3-11 and 13-18* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1, 3-11 and 13-18* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Applicants' amendment filed on August 26, 2002 in Paper No. 8 has been entered.

Amended claims 1, 3-11 and 13-18 are pending in the present application.

The text of those sections of Title 35 U.S.C. Code not included in this action can be found in a prior Office Action.

Response to Amendment

The rejection under 35 U.S.C. 102(a) as being anticipated by Su-Chun Zhang et al. (Poster in Keystone Symposium on Pluripotent Stem Cells, 2/6/2001) is withdrawn in light of Applicants' Declaration.

The rejection under 35 U.S.C. 102(e) as being anticipated by Luskin (U.S. 6,251,669) as evidenced by Sandberg et al. (US2002/0028510A1) is withdrawn in light of Applicants' amendment.

Claim Objections

Claim 18 is objected to under 37 CFR 1.75 as being a substantial duplicate of claim 6. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k).

Following is a new ground of rejection necessitated by Applicants' amendment.

Claim Rejections - 35 USC § 112

Amended claims 1, 3-11 and 13-18 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In amended claims 1, 14 and their dependent claims, it is unclear what is encompassed by the phrase "cells are characterized by rosette formations", and thus it renders the claims indefinite. This is because it is unclear whether the cells have the ability to form rosette formations or actually form rosette formations. Thus, the metes and bounds of the claims are not clearly determined.

Amended claim 3 recites the limitation "central neuroepithelial islands" in lines 4-5 of the claims. There is insufficient antecedent basis for this limitation in the claim. What is the connection between the central neuroepithelial islands with neural precursors recited in the claim? Additionally, it is still unclear what is encompassed by the phrase "differential enzymatic treatment and adhesion". The enzymatic treatment and adhesion are differential in which manner and between which cell populations in the claimed method. The metes and bounds of the claim are not clearly determined.

Claim Rejections - 35 USC § 102

Amended claims 1, 4-6, 10-11, 13-16 and 18 are rejected under 35 U.S.C. 102(e) as being anticipated by Carpenter (WO 01/88104 with an international filing date of 5/16/01 with U.S. priority date to 5/17/2000; IDS).

Amended claims 1, 4-6, 10-11, 13 and 18 are drawn to a method of differentiating primate embryonic stem cells into neural precursor cells, comprising the steps of: (a) obtaining a primate embryonic stem cell culture, (b) propagating the stem cells wherein embryoid bodies are formed, and (c) culturing the embryoid bodies in a medium containing an effective amount of fibroblast growth factor 2, wherein neural precursors are generated and wherein the neural precursor cells are characterized by rosette fomations; the same method with various limitations recited in the dependent claims. Claims 14-16 are directed to an isolated cell population comprising at least 72% neural precursor cells wherein the cells are characterized by rosette formation; the same wherein the population comprises at least 84% or at least 90% neural precursor cells.

Carpenter discloses methods for culturing stem cell populations in a cocktail of growth conditions that initiates differentiation and establishes the neural progenitor population. Carpenter teaches that primate Pluripotent stem cells (pPS cells) including human, rhesus and marmoset embryonic stem cells of Thomson et al. as well as human embryonic germ cells of Shamblott et al. and other types of pluripotent cells are maintained either on irradiated primary mouse embryonic fibroblasts or in a feeder-free system, and expanded by serial passaging (pages 8-10, under "Sources of stem cells"; page 19, lines 13-22). Embryoid bodies (Ebs) are produced from the propagated

human embryonic stem cells (e.g., hES cell lines used such as H1, H9, H13, H7NG) and they are cultured in non-adherent cell culture plates in a medium composed of 80% KO DMEM and 20% non-heat-inactivated FBS supplemented with 1% non-essential amino acids. After 4-8 days in suspension, Ebs are plated onto a substrate and allowed to differentiate into neural precursors in the presence of selected differentiation factors (page 19, lines 23-31). One of the disclosed set of conditions includes the incubation of Ebs onto fibronectin in DMEM/F12 with N2 and B27 supplemented with 10 ng/mL human EGF, **10ng/mL human bFGF**, 1 ng/mL human IGF-1, and 1 ng/mL human PDGF-AA. After 2-3 days in these conditions, 25-66% of the cells express A2B5. This population is enriched by magnetic bead sorting to **48-93%** purity (example 2 and Table 4). Under another set of conditions, after culturing for about 2-3 days **25-72%** of the cells express NCAM (example 1 and Table 3). Carpenter further teaches that upon plating out the embryoid bodies onto the substrate without dispersing the cells, neural cell precursors migrate out of the embryoid bodies and on to the extracellular matrix. Subsequent passaging of these cultures into an appropriate medium helps select out the neural progenitor cells, and that as many as 30%, 50%, 75% or more of the cells prepared by the disclosed procedures express either **polysialylated NCAM or the A2B5 epitope or both**, and that these cells have the capacity to form cells of the neuronal lineage and the glial lineages (page 11, lines 34-43). Additionally, Carpenter discloses that neural stem cells can be cultured with a medium comprising glucose, transferrin, insulin, selenium, progesterone, and several other growth factors as taught in U.S. Patent No. 5,968,829 (page 8, lines 34-36). It is also noted that the referred

medium also contains heparin and putrescine. Since the method steps and starting materials (primate embryonic stem cells, cultured media and conditions) taught by Carpenter are the same as those disclosed by the presently claimed invention, the neural precursor cells of Carpenter are also capable of forming rosette formations in the cell cultures.

Accordingly, Carpenter anticipates the instant claims.

Response to argument

Applicants' argument related to the above rejection in the Amendment filed on August 26, 2002 in Paper No. 8 (pages 4-6) have been fully considered.

Applicants argue mainly that the neural precursor cells that express neural cell adhesion molecule (NCAM or polysialylated NCAM) of Carpenter are at a later stage of development and have a limited differentiation potential compared to the neural precursor cells in rosette formations of the instant invention. Applicants' argument is respectfully found to be unpersuasive for the following reasons.

Firstly, Examiner notes that the vast majority of neural precursor cells in rosette formations (or rosette cells) are also stained positive for PSA-NCAM (polysialyated NCAM) as the neural precursor cells of Carpenter (see instant specification on page 10, bottom of paragraph 22). Applicants further teach to isolate neural precursor cells of the presently claimed invention by an immune separation using an antibody to PSA-NCAM (see last paragraph on page 7 of the specification).

Secondly, since the method steps and starting materials (primate embryonic stem cells, cultured media and conditions) taught by Carpenter are the same as those disclosed by the presently claimed invention, the neural precursor cells of Carpenter are also capable of forming rosette formations in the cell cultures, and have the same differentiating potentials. Carpenter even teaches that the neural precursor cells prepared by the disclosed procedures have the capacity to form cells of the neuronal lineage and the glial lineages (page 11, lines 39-43; and see examples)

Thirdly, Applicants have failed to provide any factual evidence indicating that the neural precursor cells of Carpenter do not have the same differentiating potentials as the rosette cells of the presently claimed invention.

Accordingly, amended claims 1, 4-6, 10-11, 13-16 and 18 are rejected for the reasons set forth above.

Claim Rejections - 35 USC § 103

Amended claims 1, 6-7 and 14-17 are rejected under 35 U.S.C. 103(c) as being unpatentable over Carpenter (WO 01/88104 with an international filing date of 5/16/01 with U.S. priority date to 5/17/2000; IDS).

Amended claims 1 and 6-7 are drawn to a method of differentiating primate embryonic stem cells into neural precursor cells as recited in claim 1, wherein the percentage of neural precursor cells in the culture step (c) is at least 84%. Claims 14-17 are directed to an isolated cell population comprising at least 72% neural precursor

cells; the same wherein the population comprises at least 84% or at least 90% neural precursor cells.

Carpenter discloses methods for culturing stem cell populations in a cocktail of growth conditions that initiates differentiation and establishes the neural progenitor population. Carpenter teaches that primate Pluripotent stem cells (pPS cells) including human, rhesus and marmoset embryonic stem cells of Thomson et al. as well as human embryonic germ cells of Shamblott et al. and other types of pluripotent cells are maintained either on irradiated primary mouse embryonic fibroblasts or in a feeder-free system, and expanded by serial passaging (pages 8-10, under "Sources of stem cells"; page 19, lines 13-22). Embryoid bodies (Ebs) are produced from the propagated human embryonic stem cells (e.g., hES cell lines used such as H1, H9, H13, H7NG) and they are cultured in non-adherent cell culture plates in a medium composed of 80% KO DMEM and 20% non-heat-inactivated FBS supplemented with 1% non-essential amino acids. After 4-8 days in suspension, Ebs are plated onto a substrate and allowed to differentiate into neural precursors in the presence of selected differentiation factors (page 19, lines 23-31). One of the disclosed set of conditions includes the incubation of Ebs onto fibronectin in DMEM/F12 with N2 and B27 supplemented with 10 ng/mL human EGF, **10ng/mL human bFGF**, 1 ng/mL human IGF-1, and 1 ng/mL human PDGF-AA. After 2-3 days in these conditions, 25-66% of the cells express A2B5. This population is enriched by magnetic bead sorting to **48-93%** purity (example 2 and Table 4). Under another set of conditions, after culturing for about 2-3 days **25-72%** of the cells express NCAM (example 1 and Table 3). Carpenter further teaches that upon

plating out the embryoid bodies onto the substrate without dispersing the cells, neural cell precursors migrate out of the embryoid bodies and on to the extracellular matrix. Subsequent passaging of these cultures into an appropriate medium helps select out the neural progenitor cells, and that as many as 30%, 50%, 75% or more of the cells prepared by the disclosed procedures express either **polysialylated NCAM or the A2B5 epitope or both**, and that these cells have the capacity to form cells of the neuronal lineage and the glial lineages (page 11, lines 34-43). Since the method steps and starting materials (primate embryonic stem cells, cultured media and conditions) taught by Carpenter are not distinguishable from those of the presently claimed invention, the neural precursor cells of Carpenter are also capable of forming rosette formations in the cell cultures. Carpenter does not specifically teach that at least 84% of the cells in the cultures of embryoid bodies with the presence of bFGF are neural precursors or an isolated cell population comprising at least 95% neural precursor cells.

However, it would have been obvious and within the scope of skills for an ordinary skilled artisan to obtain the same recited percentages of neural precursor cells that are differentiated from embryoid bodies using the method taught by Carpenter by incubating the Ebs in the presence of selected differentiation factors (e.g., bFGF among others) for a longer period of time. This is because Carpenter merely exemplified that 25-66% of the cells express A2B5 (indicating neural precursors) and 25-72% of the cells express NCAM (indicating neural precursors) after 2-3 days of incubation under differentiating conditions in the presence of bFGF, and that the percentage of differentiated neural precursors would be increased upon a prolonged culture under

differentiating conditions. As such, an isolated cell population comprising at least 95% neural precursor cells would also be obtained via various means of further purification approaches taught by Carpenter.

Accordingly, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Response to argument

Applicants' arguments related to the above rejection in the Amendment filed on August 26, 2002 in Paper No. 8 (page 6) have been fully considered.

Applicants argue basically that Carpenter does not disclose neural precursor cells characterized by rosette formation and that the neural precursor cells that express neural cell adhesion molecule (NCAM) of Carpenter are at a later stage of development and have a limited differentiation potential compared to the neural precursor cells in rosette formations of the instant invention. Applicants' arguments are respectfully found to be unpersuasive for the same reasons already set forth in the previous Response with regarding to the rejection of claims 1, 4-6, 10-11, 13-16 and 18.

Conclusions

No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP

§ 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (703) 308-8339.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's mentor, Dave Nguyen, may be reached at (703) 305-2024, or SPE, Irem Yucel, Ph.D., at (703) 305-1998.

Any inquiry of a general nature or relating to the status of this application should be directed to LIE, Tiffany Tabb, whose telephone number is (703) 605-1238.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1636.

Quang Nguyen, Ph.D.

JAMES KETTER
PRIMARY EXAMINER